growth factor and betacellulin. Ligand binding induces a variety of cellular responses including mitogenesis and differentiation. Multiple proteolytic events allow for the release of a cytoplasmic fragment and an extracellular fragment. Mutations in this gene have been associated with cancer. Alternatively spliced variants which encode different protein isoforms have been described; however, not all variants have been fully characterized.

Anti-HER4 antibodies for use in anti-cancer therapy are known e.g. from US 5,811,098, US 7,332,579 or Hollmén M, et al, Oncogene. 28 (2009) 1309-19 (anti-ErbB-4 antibody mAb 1479).

So far it was not possible to select antigen binding proteins like e.g. antibodies that specifically bind to the beta-hairpin of HER3 and/or HER4 as these beta-hairpins of HER3 or of HER4 both represent hidden epitopes, which are not accessible in the equilibrium state of these receptors (see Figure 1).

Summary ~~of the Invention~~

We now have found a method using the beta-hairpins of HER3 and HER4 functionally presented in a 3-dimensional orientation within SlyD scaffolds (see e.g Figure 2, and the polypeptides of SEQ ID NOs. 13, and 17 to 24) to obtain such antigen binding proteins, in particular antibodies.

The ~~invention~~ disclosure provides, in one aspect, a method for selecting an antigen binding protein, in particular an antibody that binds to human HER3 and binds to human HER4,

wherein the antigen binding protein, in particular the antibody, binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) of human HER3 and binds within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) of human HER4;

wherein

a) at least one polypeptide selected from the group consisting of:

SEQ ID NO: 13 TtSlyD-FKBP-Her3,
SEQ ID NO: 17 TtSlyDcas-Her3,
SEQ ID NO: 18 TtSlyDcys-Her3,
SEQ ID NO: 19 TgSlyDser-Her3, and
SEQ ID NO: 20 TgSlyDcys-Her3,

which comprises the amino acid sequence of SEQ ID NO:1;
and

b) at least one polypeptide selected from the group consisting of:

SEQ ID NO: 21 TtSlyDcas-Her4,
SEQ ID NO: 22 TtSlyDcys-Her4,
SEQ ID NO: 23 TgSlyDser-Her4, and
SEQ ID NO: 24 TgSlyDcys-Her4,

which comprises the amino acid sequence of SEQ ID NO:2;

are used to select antigen binding proteins, in particular antibodies, which show binding to both, the at least one polypeptide under a) and the at least one polypeptide under b)

and thereby selecting an antigen binding protein, in particular an antibody, that binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) and within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2).

The disclosure ~~invention~~ provides, in another aspect, an antigen binding protein, in particular an antibody, obtained by such selection method.

The ~~invention~~disclosure provides, in another aspect, an isolated antigen binding protein, in particular an antibody, that binds to human HER3 and binds to human HER4, wherein the antigen binding protein, in particular the antibody, binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) of human HER3 and binds within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) of human HER4.

The ~~invention~~disclosure provides an isolated antigen binding protein that binds to human HER3 and that binds to human HER4,

a) wherein the antigen binding protein binds to a polypeptide of

SEQ ID NO: 18 TtSlyDcys-Her3,

and

b) wherein the antigen binding protein binds to a polypeptide of

SEQ ID NO: 22 TtSlyDcys-Her4.

The ~~invention~~disclosure further provides an isolated antigen binding protein that binds to human HER3 and that binds to human HER4,

a) wherein the antigen binding protein binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide of SEQ ID NO: 18 (TtSlyDcys-Her3), and

b) wherein the antigen binding protein binds within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide of SEQ ID NO: 22 (TtSlyDcys-Her4).

The ~~invention~~disclosure provides an isolated antibody that binds to human HER3 and that binds to human HER4,

a) wherein the antibody binds to a polypeptide of

SEQ ID NO: 18 TtSlyDcys-Her3,

and

b) wherein the antibody binds to a polypeptide of

SEQ ID NO: 22 TtSlyDcys-Her4.

The ~~invention~~disclosure further provides an isolated antibody that binds to human HER3 and that binds to human HER4,

a) wherein the antibody binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide of SEQ ID NO: 18 (TtSlyDcys-Her3), and

b) wherein the antibody binds within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide of SEQ ID NO: 22 (TtSlyDcys-Her4).

The ~~invention~~disclosure provides, in another aspect, an isolated antibody that binds to human HER3 and that binds to human HER4, wherein the antibody has one or more of the following properties:

a) the antibody binds to the amino acid sequence of SEQ ID NO:1; and/or

b) the antibody binds to the amino acid sequence SEQ ID NO:1 in activated HER3; and/or

c) the antibody binds within an amino acid sequence of PQPLVYNKLTFQLEPNPHT (SEQ ID NO:1) which is comprised in a polypeptide selected from the group consisting of:

SEQ ID NO: 13	TtSlyD-FKBP-Her3,
SEQ ID NO: 17	TtSlyDcas-Her3,
SEQ ID NO: 18	TtSlyDcys-Her3,
SEQ ID NO: 19	TgSlyDser-Her3, and
SEQ ID NO: 20	TgSlyDcys-Her3;

and/or

d) the antibody binds to the ß-hairpin region of HER3; and/or

e) the antibody inhibits the heterodimerisation of HER3/HER2 heterodimers; and/or

f) the antibody binds to HER3-ECD with a ratio of the association constant (Ka) in presence of Heregulin (Ka (+Heregulin)) and in absence of Heregulin (Ka (-Heregulin)) of 4.0 or higher (Ka (+Heregulin))/(Ka (-Heregulin)); and/or

g) the antibody binds to HER3-ECD with a ratio of the Molar Ratio MR of binding in presence of Heregulin (MR (+Heregulin)) and in absence of Heregulin (MR (-Heregulin)) of 2.0 or higher (MR (+Heregulin))/ (MR (-Heregulin)); and/or

h) the antibody specifically binds to the amino acid sequence of SEQ ID NO:2; and/or

i) the antibody binds to the amino acid sequence SEQ ID NO:2 in activated HER4; and/or

j) the antibody binds within an amino acid sequence of PQTFVYNPTTFQLEHNFNA (SEQ ID NO:2) which is comprised in a polypeptide selected from the group consisting of:

SEQ ID NO: 21	TtSlyDcas-Her4,
SEQ ID NO: 22	TtSlyDcys-Her4,

SEQ ID NO: 23 TgSlyDser-Her4, and
SEQ ID NO: 24 TgSlyDcys-Her4;
and/or

k) the antibody binds to the ß-hairpin region of HER4; and/or

l) the antibody binds to HER4-ECD with a ratio of the association constant (Ka) in presence of Heregulin (Ka (+Heregulin)) and in absence of Heregulin (Ka (-Heregulin)) of 20.0 or higher (Ka (+Heregulin))/(Ka (-Heregulin)); and/or

m) the antibody binds to HER4-ECD with a ratio of the Molar Ratio MR of binding in presence of Heregulin (MR (+Heregulin)) and in absence of Heregulin (MR (-Heregulin)) of 5.0 or higher (MR (+Heregulin))/ (MR (-Heregulin)); and/or

n) the antibody shows as monovalent Fab fragment the same or higher biological activity as compared to its bivalent parent full length antibody; and/or

o) the antibody inhibits the HER3 phosporylation in MCF-7 cells; and/or

p) the antibody does not compete for binding to HER3 with Heregulin/ induces binding of Heregulin to HER3; and/or

q) the antibody inhibits the proliferation of MDA-MB-175 cells tumor cells; and/or

r) the antibody shows tumor growth inhibitory activity in vivo; and/or

s) the antibody binds with an affinity of a KD value $\leq 1 \times 10^{-8}$ M to HER3-ECD (in one embodiment with a KD value of 1×10^{-8} M to 1×10^{-13} M; (in one embodiment with a KD value of 1×10^{-9} M to 1×10^{-13} M); and/or

t) the antibody binds with an affinity of a KD value $\leq 1 \times 10^{-8}$ M to HER4-ECD (in one embodiment with a KD value of 1×10^{-8} M to 1×10^{-13} M; (in one embodiment with a KD value of 1×10^{-9} M to 1×10^{-13} M); and/or

u) the antibody binds to a polypeptide consisting of VYNKLTFQLEP (SEQ ID NO:43) and to a polypeptide of consisting of VYNPTTFQLE (SEQ ID NO:44); and/or

v) the antibody binds to a polypeptide consisting of VYNKLTFQLEP (SEQ ID NO:43); and/or

w) the antibody binds to a polypeptide consisting of VYNPTTFQLE (SEQ ID NO:44); and/or

x) the antibody binds in a FACS assay to HER3 expressing T47D cells; and wherein the antibody induces an at least 25% higher percentage of internalization of HER3 in the presence of Heregulin as compared to the percentage of internalization of HER3 in the presence of Heregulin when measured after 1 h after antibody exposure.

In one embodiment such anti-HER3/HER4 antibody is a monoclonal antibody.

In one embodiment such anti-HER3/HER4 antibody is a human, humanized, or chimeric antibody.

In one embodiment such anti-HER3/HER4 antibody is an antibody fragment that binds human HER3 and that binds human HER4.

The disclosure provides, in another aspect, an antibody that binds to human HER3 and that binds human HER4, wherein the antibody binds to a polypeptide with a length of 15 amino acids comprising the amino acid sequence VYNKLTFQLEP (SEQ ID NO:43) and to a polypeptide with a length of 15 amino acids comprising the amino acid sequence VYNPTTFQLE (SEQ ID NO: 44).

In one embodiment such anti-HER3/HER4 antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27.

In one embodiment such anti-HER3/HER4 antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

In one embodiment such anti-HER3/HER4 antibody comprises

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:26; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:28; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:29; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30;

ii) or a humanized variant of the HVRs of the antibody under i) (a), (b), (d) and/or (e).

In one embodiment such anti-HER3/HER4 antibody comprises

i) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:40; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:41; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

ii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:38; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:40; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

iii) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:40; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:41; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30; or

iv) (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:25; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:39; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:27; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:40; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:42; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:30.

The disclosure provides, in another aspect, an antibody comprising:

a) a VH sequence of SEQ ID NO:33 and a VL sequence of SEQ ID NO:36; or

b) a VH sequence of SEQ ID NO:33 and a VL sequence of SEQ ID NO:37; or

c) a VH sequence of SEQ ID NO:34 and a VL sequence of SEQ ID NO:36; or

d) a VH sequence of SEQ ID NO:34 and a VL sequence of SEQ ID NO:37; or

e) a VH sequence of SEQ ID NO:35 and a VL sequence of SEQ ID NO:36; or

f) a VH sequence of SEQ ID NO:35 and a VL sequence of SEQ ID NO:37.

In one embodiment such anti-HER3/HER4 antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:33; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:36; or (c) a VH sequence as in (a) and a VL sequence as in (b). In one embodiment such anti-HER3/HER4 antibody comprises a VH

sequence of SEQ ID NO:36; or (c) a VH sequence as in (a) and a VL sequence as in (b). In one embodiment such anti-HER3/HER4 antibody comprises a VH sequence of SEQ ID NO: 35. In one embodiment such anti-HER3/HER4 antibody comprises a VL sequence of SEQ ID NO: 36. In one embodiment such anti-HER3/HER4 antibody comprises a VH sequence of SEQ ID NO:35 and a VL sequence of SEQ ID NO:36.

In one embodiment such anti-HER3/HER4 antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:35; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:37; or (c) a VH sequence as in (a) and a VL sequence as in (b). In one embodiment such anti-HER3/HER4 antibody comprises a VH sequence of SEQ ID NO: 35. In one embodiment such anti-HER3/HER4 antibody comprises a VL sequence of SEQ ID NO: 37. In one embodiment such anti-HER3/HER4 antibody comprises a VH sequence of SEQ ID NO:35 and a VL sequence of SEQ ID NO:37.

In one embodiment such anti-HER3/HER4 antibody is a full length IgG1 antibody or IgG4 antibody.

In one embodiment such anti-HER3/HER4 antibody is a Fab fragment.

The ~~invention~~disclosure further provides, in another aspect, an isolated nucleic acid such anti-HER3/HER4 antibody.

The ~~invention~~disclosure further provides, in another aspect, a host cell comprising such nucleic acid.

The ~~invention~~disclosure further provides a method of producing an antibody comprising culturing such host cell so that the antibody is produced.

In on embodiment such method further comprises recovering the antibody from the host cell. Hence, the disclosure provides, in another aspect, a method of producing an antibody comprising culturing the host cell as defined herein so that the antibody is produced, and recovering said antibody from said cell culture or the cell culture supernatant.

The ~~invention~~disclosure further provides, in another aspect, an immunoconjugate comprising such anti-HER3/HER4 antibody and a cytotoxic agent.

The ~~invention~~disclosure further provides a pharmaceutical formulation comprising such anti-HER3/HER4 antibody and a pharmaceutically acceptable carrier. The disclosure provides, in another aspect, a pharmaceutical formulation comprising the antibody as defined herein, or the immunoconjugate as defined herein, and a pharmaceutically acceptable carrier.

The ~~invention~~disclosure further provides, in another aspect, the anti-HER3/HER4 antibody described herein, or the immunoconjugate comprising the anti-HER3/HER4 antibody and a cytotoxic agent, for use as a medicament. The ~~invention~~disclosure further provides, in another aspect, the anti-HER3/HER4 antibody described herein, or the immunoconjugate comprising the anti-HER3/HER4 antibody and a cytotoxic agent, for use in treating cancer. The ~~invention~~disclosure further provides, in another aspect, the anti-HER3/HER4 antibody described herein for use in inhibition of HER3/HER2 dimerization.

The disclosure further provides, in another aspect, u~~U~~se of such anti-HER3/HER4 antibody, or an immunoconjugate comprising the anti-HER3/HER4 antibody and a cytotoxic agent, in the manufacture of a medicament. Such use wherein the medicament is for treatment of cancer.Such use wherein the medicament is for the inhibition of HER3/HER2 dimerization.

The ~~invention~~disclosure further provides a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-HER3/HER4 antibody described herein, or an immunoconjugate comprising the anti-HER3/HER4 antibody and a cytotoxic agent.

The ~~invention~~disclosure further provides a method of inducing apoptosis in a cancer cell in an individual sufferning from cancer comprising administering to the individual an effective amount of an immunoconjugate comprising the anti-HER3/HER4 antibody described herein and a cytotoxic agent, thereby inducing apoptosis in a cancer cell in the individual.

The disclosure further provides, in another aspect, ~~One embodiment of the invention is~~ a polypeptide selected from the group consisting of:

i) SEQ ID NO: 13 TtSlyD-FKBP-Her3,
ii) SEQ ID NO: 17 TtSlyDcas-Her3,
iii) SEQ ID NO: 18 TtSlyDcys-Her3,
iv) SEQ ID NO: 19 TgSlyDser-Her3, and
v) SEQ ID NO: 20 TgSlyDcys-Her3,

which polypeptide comprises the amino acid sequence of SEQ ID NO:1

The disclosure further provides, in another aspect, ~~One embodiment of the invention is~~ a polypeptide selected from the group consisting of:

i) SEQ ID NO: 21 TtSlyDcas-Her4,
ii) SEQ ID NO: 22 TtSlyDcys-Her4,

iii) SEQ ID NO: 23 TgSlyDser-Her4,and
iv) SEQ ID NO: 24 TgSlyDcys-Her4,

which polypeptide comprises the amino acid sequence of SEQ ID NO:2.

The ~~invention~~disclosure further provides, in another aspect, the use of one of such polypeptides selected from the group consisting of:

i) SEQ ID NO: 13 TtSlyD-FKBP-Her3,
ii) SEQ ID NO: 17 TtSlyDcas-Her3,
iii) SEQ ID NO: 18 TtSlyDcys-Her3,
iv) SEQ ID NO: 19 TgSlyDser-Her3, and
v) SEQ ID NO: 20 TgSlyDcys-Her3,

for eliciting an immune response against SEQ ID NO:1 in an experimental animal.

The ~~invention~~disclosure further provides, in another aspect, the use of one of such polypeptides selected from the group consisting of:

i) SEQ ID NO: 21 TtSlyDcas-Her4,
ii) SEQ ID NO: 22 TtSlyDcys-Her4,
iii) SEQ ID NO: 23 TgSlyDser-Her4,and
iv) SEQ ID NO: 24 TgSlyDcys-Her4,

for eliciting an immune response against SEQ ID NO:2 in an experimental animal.

The ~~invention~~disclosure further provides a method for producing an antibody specifically binding to the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1 comprising the following steps:

a) administering to an experimental animal a polypeptide selected from the group consisting of:

i) SEQ ID NO: 13 TtSlyD-FKBP-Her3,
ii) SEQ ID NO: 17 TtSlyDcas-Her3,
iii) SEQ ID NO: 18 TtSlyDcys-Her3,
iv) SEQ ID NO: 19 TgSlyDser-Her3, and

v) SEQ ID NO: 20 TgSlyDcys-Her3,

for at least one time, whereby the polypeptide comprises the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1,

b) recovering from the experimental animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1, and

c) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1 and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

The ~~invention~~disclosure further provides the use of a polypeptide selected from the group consisting of:

i) SEQ ID NO: 13 TtSlyD-FKBP-Her3,

ii) SEQ ID NO: 17 TtSlyDcas-Her3,

iii) SEQ ID NO: 18 TtSlyDcys-Her3,

iv) SEQ ID NO: 19 TgSlyDser-Her3, and

v) SEQ ID NO: 20 TgSlyDcys-Her3,

for epitope mapping, whereby the polypeptide comprises the epitope in the the ß-hairpin of HER3 with the amino acid sequence of SEQ ID NO:1.

A method for producing an antibody specifically binding to the ß-hairpin of HER4 with the amino acid sequence of SEQ ID NO:2 comprising the following steps:

a) administering to an experimental animal a polypeptide selected from the group consisting of:

i) SEQ ID NO: 21 TtSlyDcas-Her4,

ii) SEQ ID NO: 22 TtSlyDcys-Her4,

iii) SEQ ID NO: 23 TgSlyDser-Her4,and

iv) SEQ ID NO: 24 TgSlyDcys-Her4,

for at least one time, whereby the polypeptide comprises the ß-hairpin of HER4 with the amino acid sequence of SEQ ID NO:2,

b) recovering from the experimental animal three to ten days after the last administration of the polypeptide B-cells that produce the antibody specifically binding to the ß-hairpin of HER4 with the amino acid sequence of SEQ ID NO:2, and

c) cultivating a cell comprising a nucleic acid encoding the antibody specifically binding to the the ß-hairpin of HER4 with the amino acid sequence of SEQ ID NO:2 and recovering the antibody from the cell or the cultivation medium and thereby producing an antibody specifically binding to a target antigen.

The ~~invention~~disclosure further provides the use of a polypeptide selected from the group consisting of:

i) SEQ ID NO: 21 TtSlyDcas-Her4,

ii) SEQ ID NO: 22 TtSlyDcys-Her4,

iii) SEQ ID NO: 23 TgSlyDser-Her4,and

iv) SEQ ID NO: 24 TgSlyDcys-Her4,

*for epitope mapping, whereby the polypeptide comprises the epitope in the the ß-*hairpin of HER4 with the amino acid sequence of SEQ ID NO:2.

Using the beta-hairpins of HER3 and HER4 functionally presented in a 3-dimensional orientation within SlyD scaffolds (see e.g Figure 2, and the polypeptides of SEQ ID NOs. 13, and 17 to 24) the anti-HER3/HER4 antigen binding proteins, in particular antibodies, described herein binding to these beta-hairpins could be selected. It was found that the antigen binding proteins, in particular antibodies,according to the ~~invention~~disclosure have highly valuable properties such as strong growth inhibition of HER3 expressing cancer cells, strong inhibition of HER3 mediated signal transduction (such as e.g. HER3 phoshorylation) which is related to cancer cell proliferation, or very specific pharmacokinetic properties (such as faster association rates and higher Molar Ratios of the binding the